

PRELIMINARY AMENDMENT

Serial Number: 08/012,269

Filing Date: February 1, 1993

Title: MURINE 4-1BB GENE (as amended)

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33 The amino-terminal sequence of the purified 4-1BBPs was determined. The sequence was Val-Gln-Asn-Ser-X-Asp (SEQ ID NO:6). The amino acid sequence at positions 1, 2, 3, 4 and 6 was identical to that of the mature 4-1BBP predicted from the cDNA sequence. Amino acid at position 5 which is supposed to be Cys was not determined. These results indicate that the deduced amino acid sequence and assignment of signal sequence are correct. When the potential transmembrane domain was removed from the complete 4-1BB molecule, the protein was secreted. These results suggested that 4-1BBP was likely to be associated with the cellular membrane as predicted by the primary structure.

Please substitute the third paragraph on page 37 continuing on page 38 for the paragraph in the appendix entitled "Clean Version of the Third paragraph on Page 37 Continuing on Page 38." Specific amendments to the third paragraph on page 37 continuing on page 38 are detailed in the following marked-up paragraph:

34 Antibody Preparation. An oligopeptide representing amino acids 105-115 of the deduced 4-1BBP sequence was synthesized (Applied Biosystems). The sequence was NH₂-CRPGQELTKSGY-COOH (SEQ ID NO:7). A tyrosine residue at the C-terminus of the peptide was added for possible radioactive labeling with [¹²⁵I]. The peptide was conjugated to keyhole limpet hemocyanin (KLH) with a [heterobifunctional] heterobifunctional cross linker, m-maleimidobenzoyl-n-hydroxysuccinimide ester (88, 107).

Please substitute page 39, second full paragraph for the paragraph in the appendix entitled "Clean Version of Page 39, Second Full Paragraph." Specific amendments to page 39, second full paragraph are detailed in the following marked-up paragraph:

35 This region forms the pattern of C-X₂-C-X₉-H-X₃C-X-C (SEQ ID NO:8); and the cysteines and histidine are conserved in a similar space in 4-1BB, sina, and DG17 proteins. Ten of 24 amino acids between the 4-1BB and sina proteins are identical. Between 4-1BB and DG17 proteins, 11 of 24 amino acids are identical, and 3 of 24 are conservative substitutions. The conserved pattern suggests that these amino acids are functionally important.

Please substitute page 44, first full paragraph for the paragraph in the appendix entitled "Clean Version of Page 44, First Full Paragraph." Specific amendments to page 34, first full paragraph are detailed in the following marked-up paragraph:

36 4-1BB contains other interesting features in its cytoplasmic domain. Those include
1) two runs of acidic amino acids; 2) a potential p56^{lck} binding site; 3) five consecutive

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glycines at the carboxyl terminus; and 4) four potential phosphorylation sites - 1 tyrosine, 2 threonine, and 1 serine. It is especially interesting that 4-1BB contains a potential p56^{lck} binding site, -C-R-C-P- (SEQ ID NO:9). The consensus sequence of p56^{lck} binding site is -C-X-C-P- (SEQ ID NO:10) in the CD4 and CD8 molecules (93).

Please substitute the third paragraph on page 45 continuing on page 46 for the paragraph in the appendix entitled "Clean Version of the Third paragraph on Page 45 Continuing on page 46." Specific amendments to the third paragraph on page 45 continuing on page 46 are detailed in the following marked-up paragraph:

To construct a plasmid that expresses extracellular portion of 4-1BB, the putative extracellular domain of 4-1BB cDNA (89) was amplified by polymerase chain reaction (PCR) (99). An XhoI site was created at the 5' end of the forward primer and a stop codon, (TAA), and an EcoRI site were created in the reverse primer. The PCR product was digested with XhoI and EcoRI and the -0.6 kb fragment was purified. The XhoI-EcoRI fragment (4-1BBS) was inserted into the PEV-55 vectors (53), generating PEV-55-4-1BBS. The sequence of the forward primer (SEQ ID NO:11) was 5' -ACCTCGAGGTCCTGTGCATGTGACA-3' and that of the reverse primer (SEQ ID NO:12) was 5' -ATGAATTCTTACTGCAGG-AGTGCCC-3'.

Please substitute page 58, paragraph 1 for the paragraph in the appendix entitled "Clean Version of Page 58, Paragraph 1." Specific amendments to page 58, paragraph 1 are detailed in the following marked-up paragraph:

This region forms the pattern of C-X₂-C-X₂-C-X₃-H-X₃-C-X-C (SEQ ID NO:8); and the cysteines and histidine are conserved in a similar space in 4-1BB, *sina*, and DG17 proteins. Ten of 24 amino acids between the 4-1BB and *sina* proteins are identical, and 3 of 24, are conservative substitutes. The conserved pattern suggests that these amino acids are functionally important. The *sina* protein is localized in the nucleus, suggesting that it has a regulatory function in cells. The fact that the amino acid sequence of 4-1BB contains features like a zinc finger motif, a nuclear protein, and a receptor domain suggests that 4-1BB may play diverse roles during cellular proliferation and differentiation.

Please substitute page 58, paragraph 2 for the paragraph in the appendix entitled "Clean Version of Page 58, Paragraph 2." Specific amendments to page 58, paragraph 2 are detailed in the following marked-up paragraph: